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Selective macroautophagy for immunity

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Abstract

Macroautophagy was thought to be an unspecific bulk degradation process. However, Ponpuak et al. (2010) show in this issue of Immunity that cytosolic proteins are selectively recruited to autophagosomes to become metabolized to bactericidal peptides.

Macroautophagy, one of at least three autophagic pathways that deliver cytoplasmic constituents for lysosomal degradation, has been originally characterized by its ability to prolong survival during times of starvation by recycling of cellular content for energy and macromolecular building block generation. However, multiple studies have demonstrated during the last five years that this catabolic pathway can also be used for innate and adaptive immunity to intracellular pathogens (Münz, 2009).While during starvation the choice of macroautophagic substrate might be less specific and rapid energy generation might be the main objective, immunity to intracellular pathogens by macroautophagy should obviously target these specifically for lysosomal degradation, while leaving other cytoplasmic content unperturbed. In support of this concept, selective macroautophagy was first described for the steady state degradation of mitochondria and the import of hydrolase proenzymes into lysosomes via this pathway (Kanki et al., 2009; Okamoto et al., 2009; Shintani and Klionsky, 2004). The autophagy-related gene (atgs) products 32 and 19 were identified to mediate this selective import of the respective organelles and protein aggregates into autophagosomes. Similar mechanisms were suspected to mediate selective intracellular pathogen clearance by macroautophagy. Along these lines, Terje Johansen and his colleagues identified two proteins, called p62/sequestosome 1 and neighbor of BRCA1 gene (NBR1), that could link ubiquitinated substrates to Atg8/LC3 via their ubiquitin-associated (UBA) domains and LC3-interaction regions (LIR) (Bjorkoy et al., 2005; Kirkin et al., 2009). Ubiquitinated protein aggregates had been found to accumulate in the absence of macroautophagy and in autophagosomes. In addition, Atg8/LC3 was an attractive anchor for substrate recruitment into autophagosomes, because this ubiquitin-like molecule gets coupled to phosphatidylethanolamine on both the outer and inner autophagosomal membrane during macroautophagy initiation, and remains associated with the inner membrane after autophagosome completion even in its lysosomal degradation. However, overall macroautophagic degradation of long lived proteins and autophagosome formation was not affected by loss of p62/sequestosome 1, but ubiquitinated aggregate formation was significantly impaired (Komatsu et al., 2007). This suggested that either these ubiquitin with

Atg8/LC3 bridging adaptor proteins were not important for substrate recruitment to autophagosomes, or they were redundant for overall macroautophagic flux, each recruiting just a subset of substrates. Both theories were additionally supported by the fact that NBR1 degradation by macroautophagy was indeed insensitive to p62/sequestosome 1 loss (Kirkin et al., 2009). The new study by Ponpuak and colleagues in this issue of Immunity (Ponpuak et al.) now provides evidence for the second hypothesis of selective recruitment of a subset of substrates by each of these ubiquitin binding Atg8/LC3 adaptor proteins. They demonstrate that p62/sequestosome 1 is required for the mycobactericidal activity of macroautophagy. Interestingly, p62/sequestosome 1 is not required to pull mycobacteria replicating phagosomes into macroautophagy, but instead to generate mycobactericidal activity in the fusion vesicles. A previous study had demonstrated that mycobactericidal peptides could be generated from macroautophagic cargo delivered to mycobacteria containing phagosomes (Alonso et al., 2007). In the current study, particularly Fau, a metazoan fusion protein between the ribosomal protein S30 and the ubiquitin-like domain FUb, was selectively engulfed in autophagosomes and gave rise to mycobactericidal peptides in autolysosomes (Figure 1). P62/sequestosome 1 was required for macroautophagy of Fau and bound to unmodified or monoubiquitinated Fau. In contrast to p62/sequestosome 1, NBR1 did not localize to mycobacteria containing vesicles. Thus, Fau is recruited to autophagosomes by p62/sequestosome 1, and this selective macroautophagy is required to generate mycobactericidal activity in autolysosomes. In addition to mycobacterial clearance, selective macroautophagy seems to be also required for innate immunity against cytosolic bacterial pathogens. Salmonella typhimurium gets coated with ubiquitinated proteins after release from salmonella containing vacuoles (SCVs), which leads to its recruitment to autophagosomes via nuclear dot protein 52 (NDP52) (Thurston et al., 2009). NDP52 contains, like p62/sequestosome 1 and NBR1, UBA and LIR domains and delivers gram-negative Salmonella bacteria to macroautophagy (Figure 1). Therefore, selective macroautophagy via LIR and UBA containing proteins leads to both pathogen and self-protein delivery to lysosomal degradation. Different adaptor proteins seem to recruit

distinct sets of macroautophagy substrates to autophagosomes. This allows for differential regulation of selective macroautophagy via the expression levels and post-translational modifications of these adaptor proteins. Furthermore, since ubiquitin seems to serve as a tag for selective macroautophagy substrates via recruitment of these adaptor proteins, ubiquitination can serve as another regulatory mechanism for selectivity of macroautophagy. The large number of E3 ubiquitin ligases, mediating ubiquitin conjugation to different substrates, can now also influence the recruitment of p62/sequestosome 1, NBR1, NDP52 and maybe other LIR and UBA containing proteins. This allows for two additional regulation mechanisms, in addition to overall autophagosome generation and degradation, that can influence macroautophagy of cytoplasmic constituents. Therefore, macroautophagy has come a long way from its original description as an unspecific bulk degradation pathway to a possibly fine-tuned machinery for the clearance of cytoplasmic self- and foreign-structures, including pathogens.

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Figure legend

Figure 1: Substrate recruitment for selective macroautophagy. Different ubiquitin-associated (UBA) domain and LC3-interaction region (LIR) containing proteins target different substrates to autophagosomes. P62/sequestosome bridges Fau via binding to its ubiquitin-like domain (FUb) or covalently linked ubiquitin (Ub) with Atg8/LC3, which is coupled to the autophagosomal membrane. This is required for macroautophagy of Fau and generation of Fau derived bactericidal peptides. Cytosolic bacteria like Salmonella, on the other hand, get coated with ubiquitinated proteins, which are then recognized by NDP52. NDP52 binding to Atg8/LC3 delivers these bacteria then to autophagosomes and successive degradation after fusion with lysosomes.

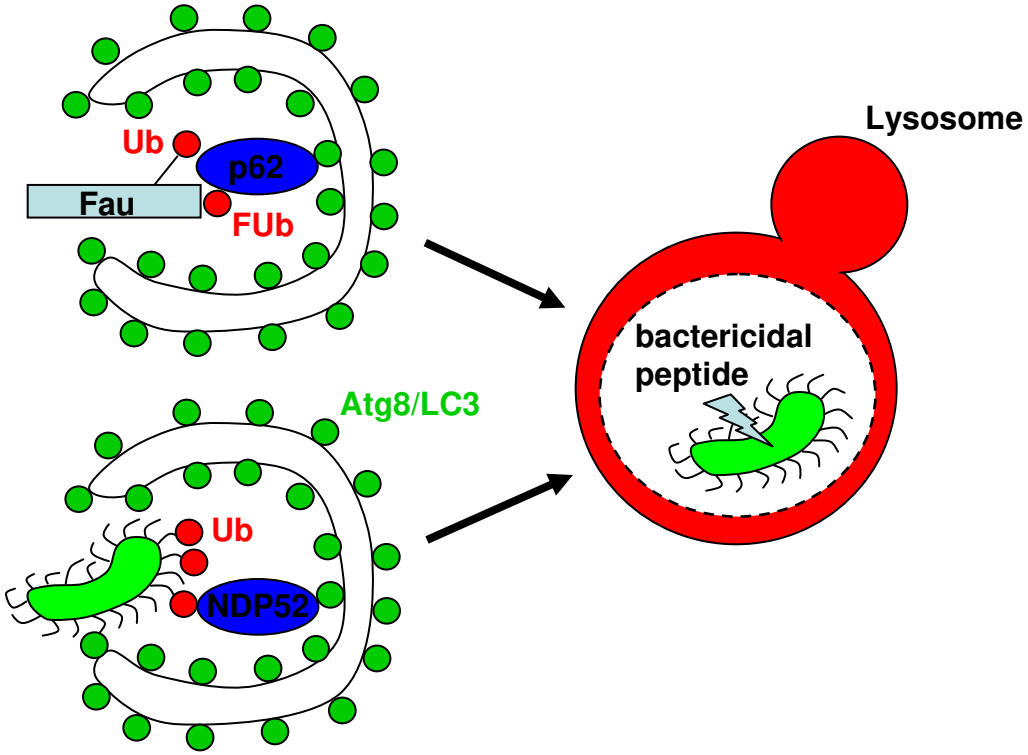
Ub
p62
Fau
FUb
Atg8/LC3
Ub
NDP52
Lysosome
bactericidal
peptide

Figure 1